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COMPLETE SPECIFICATION

Carotenoid Recovery

I, MARIA ANNA MORICZ-SMITH, Citizen of the United States of America, residing at Apt. 6F., 621 Avenue Z., Brooklyn, 23, New York, State of New York, United States of America, do hereby declare the invention, for which I pray that a patent may be granted to me, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a method for the recovery of valuable naturally occurring substances from plant sources, particularly to a method for the extraction and separation into valuable fractions of products occurring naturally in the seed pulp of *Bixa orellana*, and to the fractions and products obtained thereby.

It is known that *Bixa orellana*, and certain related species of the order Bixaceae which are indigenous to Central America and adjacent regions, contain valuable substances of the carotenoid group. These compounds occur principally in the fleshy pulp surrounding the seed. The principal carotenoids present in the seed pulp of *Bixa orellana* are *cis*- and *trans*-bixin, which are members of the polyene acid group of carotenoids, although they are associated in the pulp with varying proportions of other polyene hydrocarbon, polyene ester, polyene alcohol and polyene ketons carotenoids, as well as with certain wax pigments and other substances. Commercial products have been prepared from the seed pulp of *Bixa orellana* by various methods and are available to a limited extent in commerce. These products have, however, not been standardized and the methods used in preparing them are highly empirical and inefficient and do not give rise to products in which the individual compounds can be used to best advantage.

Applicant is not aware of any successful attempt which has been made heretofore to separate the constituents of *Bixa orellana* into a plurality of fractions, each better adapted for a specific use than the others. Thus, it is known that, whereas some of the extractable constituents of the pulp exhibit a definite biological activity in increasing the vitamin A level and pigmentation in animal or anisms, this is not particularly true of *cis*-bixin, which is one of the principal constituents of the pulp. On the other hand, *cis*-bixin itself is a valuable coloring agent for foodstuffs and is much superior to some of the other constituents of the pulp for this purpose. It is thus apparent that for best utilization of the product a separation of the constituents of the pulp into fractions, each of which is better adopted to a specific use than are the other fractions, is desirable. However, in spite of the unstandardized nature of the products heretofore available, they have found considerable use as coloring agents for foodstuffs. They have also been investigated to some extent for use in medicine. However, the use of these products has not developed to any great extent in spite of their known potential value.

The methods utilized heretofore in recovering products from the seed pulp of *Bixa orellana* in marketable form have invariably been characterized by a lack of appreciation of the nature and possible utility of individual compounds, or of particular groups of compounds, present in the pulp and have failed to take into account the factors which lead either to a maximum yield of the desired products or to the preparation of products consisting of individual compounds or mixtures of compounds best adapted to specific purposes. It is known that certain of the constituents of the seed pulp of *Bixa orellana* can be extracted with such organic solvents as chloroform, ethyl acetate or acetic acid, but such methods as practised heretofore have generally effected only a partial recovery of valuable constituents of the pulp and, insofar as is known, the extract has been merely evaporated to obtain a crude

residue containing all of the compounds occurring in the pulp which are soluble in the particular solvent employed, no successful method having been arrived at for separating the crude mixture into fractions of either pure compounds or of mixtures which are more desirable individually than is the crude residue.

It has also been the practice to remove the pulp from the fruit by hand and to ferment a mixture of the pulp and water. The water-insoluble portion of the mixture is then separated and mascerated into a paste with oils or fats of various sorts. The resulting products are known variously in the trade as "annato paste" or "pate' de roncou." It is apparent that these products are of a very crude nature and that it is substantially impossible to standardize them either as to coloring effect or biological activity. Furthermore, they contain all of the dirt and other foreign substances associated with the seeds which are not converted to water-soluble substances by the fermentation step. Any of the valuable constituents of the pulp which are converted by the fermentation to water-soluble substances are, of course, lost. The general procedure for preparing this type of product also leaves much to be desired from the point of view of sanitation. In addition, no attempt is generally made to prevent oxidation and the character of the product is likely to be changed to a considerable extent.

In still another process which has been proposed and utilized to some extent for recovering valuable products from the seed pulp, the pulp is extracted with an aqueous alkali. Although this procedure is effective in extracting the *cis*- and *trans*-bixins which are polyene acids, the carotenoids which are non-acid in character, and most of the wax pigments, are either insoluble in aqueous alkali and are thus rejected with the insoluble material and lost or, if dissolved, are oxidized or otherwise decomposed rapidly and destroyed. Furthermore, the bixins themselves are somewhat unstable in alkaline solution and, under the best of conditions, considerable deterioration of both the *cis*- and *trans*-bixins occurs during the extracting, filtering and subsequent steps of recovering the bixins from the alkaline solution. The process effects no separation of the *cis*- and *trans*-bixins from one another.

Other processes which have been proposed from time to time for recovering valuable products from *Bixa orellana* are even less advantageous in the way of economy and in the preparation of desirable products than those just mentioned and have not been used to any appreciable extent. One of the principal difficulties encountered in all of the heretofore known processes involving extraction is the very low degree of solubility of the bixins in the organic liquids which have been used. This necessitates the use, and recovery, of inordinately large quantities of solvents, this being a major factor in the cost of operation of such heretofore known processes. In addition, no solvent has heretofore been found in which the *cis*- and *trans*-bixins are adequately soluble for convenient use and which can be used to effect an economical separation of the *cis*- and *trans*-bixins from one another by crystallization procedures. It is apparent that the present state of the art, insofar as the recovery of valuable products occurring naturally in the seed pulp of *Bixa orellana* in good yield and in a form more suitable than that of a simple crude extraction residue, leaves much to be desired and that the provision of such method, as well as of new and more desirable fractions of such extraction products, would be of great value in furthering the economical preparation and uses of these products. It is equally apparent that the provision of a solvent having a high solvent power for *cis*- and *trans*-bixins, and which would permit of easy separation of these compounds from one another by crystallization therefrom, would also be of great value.

It has now been found that the carotenoids and wax pigments occurring in the dried pulp of the fruits of *Bixa orellana* can be separated into a plurality of fractions, certain of which have not been available heretofore, by taking advantage of the unexpected differential solubility of certain groups of the compounds in certain classes of organic solvents and in certain mixtures thereof. Thus, it has been found that, using the method of the invention which will be described, it is feasible and economical to recover substantially all of the *cis*-bixin occurring in the pulp in the form of deep violet crystals having a purity of 90 per cent or better. It appears that this product is substantially lacking in the type of biological activity generally associated with crude extracts of *Bixa orellana*. This product is, for the sake of convenience, sometimes referred to herein as the "*cis*-bixin fraction."

In addition to substantially pure *cis*-bixin, the method of the invention provides a ready means for preparing a viscous liquid oleoresinous fraction of the extractable substances in the pulp which is substantially free of both *cis*- and *trans*-bixins. This product, which has not been described previously, is further characterized by its fruity odor and by its solubility in both polar and non-polar organic liquids. There are indications that it is biologically active in raising the vitamin A level and in promoting pigmentation in animal organisms. The product is capable of easy standardization as to coloring value. It is of considerable value in the coloring of foodstuffs and in medicinal preparations.

This product is, for convenience, herein sometimes referred to as an "oleoresinous fraction."

The method of the invention furnishes a ready means for the preparation of still a third fraction of extractable constituents of the seed pulp. This third fraction, in contrast to the viscuous liquid nature of the oleoresinous fraction, is in the form of a sticky resinous-appearing semi-solid, hereinafter frequently referred to simply as a "resinous solid," which is low in *cis*-bixin content but which contains substantially all of the *trans*-bixin extracted from the pulp. This product is soluble in cold polar organic liquids, but is substantially insoluble in non-polar organic liquids. This product, which, for convenience, is herein sometimes referred to as a "resinous fraction," also gives indications of biological activity in increasing the vitamin A level and in promoting pigmentation in animal organisms. It is of value as a coloring agent. Like the oleoresin, it can be standardized readily. This product has not been described previously.

It is apparent that the three products just described, which constitute specific fractions of material extractable from the seed pulp of *Bixa orellana*, are better adapted to specific uses than is the crude extract of the pulp prepared by hitherto known processes. Thus, it is apparent that where the coloring value of *cis*-bixin is desirable but where biological activity is of no importance or is undesirable, the availability of a pure form of *cis*-bixin, undiluted and uncontaminated by any other of the compounds extractable from the pulp, is of infinitely more value from both the economic and the utility standpoints than is the crude extract itself. On the other hand, when it is desired to investigate the biological properties of certain of the components extractable from the pulp, the provision by the method of the invention of the new oleoresinous liquid fraction and of the new resinous solid fraction, in which this activity appears to be concentrated, makes it possible to secure the desired result without the necessity of wasting a corresponding amount of the valuable *cis*-bixin. The oleoresinous and resinous fractions appear to have biological values somewhat different from one another and all three of the fractions have coloring values differing from one another. It is thus possible to compound in desired proportions any two, or all three, of the fractions produced by the method and to thus produce a composition meeting a specific need, such as a desired color shade between light yellow and deep red, in even more precise manner than it may be met by any one of the fractions alone. An additional important feature is the fact that it now becomes possible to choose a fraction of the crude extract for use in a specific instance with due regard to the solubility characteristics desired in the particular instance concerned.

Although the present invention is concerned primarily with the method for producing the *cis*-bixin fraction, the oleoresinous fraction and the resinous fraction, and with the latter two fractions as new compositions, it is pointed out that, if desired, both the oleoresinous and the resinous fractions can, by further treatment involving extraction, crystallization or distillation, or combinations thereof, be converted into still other products, including pure compounds and mixtures, which are of considerable and even more specific value. The invention in its broadest aspects contemplates such further treatments and such of the products thus produced as have not been described previously.

The process of the invention is based, in part, upon the discovery that, although substantially the entire carotenoid and wax pigment content of the pulp of the dry, ripe seeds of *Bixa orellana* is soluble in hot polar organic liquids, only a portion of these substances is soluble in non-polar organic liquids, particularly in aliphatic hydrocarbons, while a further portion is insoluble both in non-polar and in cold polar organic liquids. It is thus possible, by taking advantage of these differential solubilities, not only to extract all of the carotenoid and wax pigment components of the seed pulp, but also to separate them practically quantitatively into the three fractions heretofore described. It is to be noted that the process differs from certain heretofore described extractive processes as applied to the seed pulp of *Bixa orellana* in that not only are the carotenoid and wax pigment substances recovered substantially completely from the seed pulp, but their separation into desirable fractions is accomplished without the necessity of wasting or discarding any of these substances except such mechanical losses as may be encountered in the normal operation of such processes.

The invention is also based, in part, upon the discovery that *cis*- and *trans*-bixin are more soluble in mixtures of alkanols and haloaliphatic hydrocarbons, e.g., in a mixture of equal parts of ethanol and trichloroethylene, than in either of the substances alone and, furthermore, that *cis*-bixin can be crystallized substantially free of *trans*-bixin from a solution of the two substances in such a mixture. This is true even when the solution contains a part or all of the other substances extractable by the mixture from the seed pulp of *Bixa orellana*.

The term "polar" as used herein when referring to organic liquids is used in its accepted sense and includes such substances as the aliphatic alcohols, the ketones, and the

aliphatic acids. The term "non-polar" as used herein when referring to organic liquids is also used in its commonly accepted sense and includes such substances as the aliphatic hydrocarbons and the haloaliphatic hydrocarbons. The polar organic liquids preferred for use in the process of the invention are the aliphatic alcohols, such as methanol, ethanol and butanol, which can frequently be used alone but which are preferably used in admixture, at least, in certain steps of the process, with certain of the non-polar liquids, notably with haloaliphatic hydrocarbons such as trichlorethylene, ethylene dichloride, chloroform and propylene dichloride. Although the aliphatic acids and the aliphatic ketones can frequently be used in certain steps of the process, there is some danger that these may react with certain of the carotenoids or pigments to give undesirable products. This is particularly true of the ketones when more than slightly elevated temperatures are employed. The employment of a mixture of ethanol or other aliphatic alcohol with a haloaliphatic hydrocarbon, rather than the use of the alcohol alone, is especially advantageous in that the mixture has more advantageous solubility characteristics than does the pure alcohol. The preferred non-polar organic liquids which are employed in certain steps of the process unmixed with a polar liquid are the aliphatic hydrocarbons including pentane, hexane and decane. These are usually employed in the form of commercially available mixtures commonly sold as "petroleum ether" or "naptha."

Because of the heat sensitivity of some of the extractable products occurring in the seed pulp, and because certain steps of the process involve the evaporation of a polar or non-polar organic liquid, or both, from a solution of a part or all of the extractable components of the pulp, it is preferred to employ polar and non-polar organic liquids which do not boil above about 100 degrees centigrade. It is, of course, possible to operate the evaporative steps of the process under vacuum and to then employ liquids boiling under atmospheric pressure somewhat higher than 100 degrees centigrade, provided they do not boil above this temperature at the pressure used. Generally speaking, however, most of the process steps can be carried out conveniently under atmospheric pressure when a suitable liquid is employed.

The process of the invention is advantageously, although not necessarily always, as will be pointed out later, carried out by first suitably extracting the dry seed pulp of *Bixa orellana*, conveniently without removal of the pulp from the seeds with a suitable polar organic liquid to recover substantially all of the carotenoids and wax pigments in the pulp. The extraction can be carried out batch-wise or continuously in any suitable manner using conventional procedures. Suitable polar organic liquids which can be employed include ethanol, propanol, methanol and acetone, either individually or in admixture with one another or with non-polar organic liquids, e.g., with haloaliphatic hydrocarbons, such as chloroform, trichloroethylene and propylene dichloride. Except when a ketone is used, extraction is generally carried out at a temperature below about 100 degrees centigrade, preferably at a temperature between about 50 degrees and about 90 degrees centigrade. At temperatures above 100 degrees centigrade, considerable decomposition of some of the valuable constituents of the pulp is liable to occur while at temperatures below about 50 degrees centigrade, incomplete extraction of the valuable constituents is likely to occur unless an excessive quantity of the extracting liquid is used. In the event a ketone is used, the extracting temperature should not exceed 30 degrees to 40 degrees centigrade to avoid the production of undesirable condensation products.

The solution thus obtained is clarified by filtering while still hot and the insoluble matter is discarded. The filtered solution is then preferably evaporated to remove substantially all of the solvent and a crude extract is obtained which is further separated into fractions by the procedures to be described. The crude extract is usually in the form of a dark red or violet, viscous, partially crystalline mass. The amount of crude extract, after freeing it from solvent, generally amounts to from 6.0 to 6.5 per cent of weight of the dry seeds employed although this may vary somewhat, depending upon the actual moisture content of the pulp as well as upon the age of the seed used. Generally speaking, seeds which have been in storage for more than about one year yield only about half as much extract as fresh dry seeds. It is to be noted that the extraction is carried out without grinding or crushing the seeds and that, for this reason, the crude extract contains very little of the oils or fats present within the seed kernel itself.

The crude extract obtained as just described is generally next extracted with an unheated non-polar organic liquid to dissolve the constituents soluble therein and the solution thus obtained is separated from the insoluble constituents, e.g., by decanting or in any other suitable manner. The insoluble constituents are reserved for further treatment and the solution is heated to volatilize the non-polar liquid, the heating being carried out so that the residue is kept at a temperature below about 100 degrees centigrade, preferably at a temperature of from about 70 degrees to about 90 degrees centigrade. The

final stages of the volatilization can be carried out *in vacuo* if desirable or necessary. The still residue thus obtained, which generally amounts to approximately half of the weight of the crude extract from which it is derived, is in the form of a viscous oleoresinous liquid having a pleasant fruity odor and showing little or no tendency to crystallize after long standing. It is deep orange red in color, possesses a certain amount of biological activity and is useful for coloring purposes. The product is soluble in polar and non-polar organic liquids and is substantially free of both *cis*- and *trans*-bixins.

The non-polar liquid employed in separating the oleoresinous fraction from the crude extract is preferably an aliphatic hydrocarbon or a commercial mixture thereof, such as that boiling from about 70 degrees to about 80 degrees centigrade and often designated as "petroleum ether." It is preferable in this step of the process to avoid the use of certain non-polar organic liquids, such as the haloaliphatic hydrocarbons, because of their somewhat better solvent action on certain constituents of the crude extract which are substantially insoluble in the aliphatic hydrocarbons and which it is desirable to segregate into one of the other fractions of the crude extract to be described. The extraction of the crude extract with the aliphatic hydrocarbon can be carried out in any convenient manner but it has been found suitable to agitate the crude extract thoroughly with three or four successive portions of the hydrocarbon and to separate the insoluble material in each case by allowing it to settle and then decanting the clear liquid. The separation can be effected by filtering, if desired, but this is not always accomplished readily due to the somewhat tacky consistency which the insoluble material is likely to have. The extraction is generally carried out at ambient temperature, although it can be carried out with satisfaction at temperatures somewhat above or somewhat below ambient temperature.

The residue which is insoluble in aliphatic hydrocarbons, and similar products obtained by variations of the above procedure, as will be described subsequently, is dissolved in a hot polar solvent and the solution allowed to crystallize. It has been found advantageous in this step of the process to employ an alcohol preferably ethanol, as the polar organic liquid and to use it in admixture with a haloaliphatic hydrocarbon. Satisfactory results have been obtained using a mixture of approximately equal parts by volume of ordinary commercial 95 per cent ethanol and trichloroethylene. However, mixtures of other alcohols, such as methanol, butanol and propanol, with other haloaliphatic hydrocarbons, such as carbon tetrachloride, chloroform, ethylene dichloride and propylene dichloride, can be employed if desired. Generally speaking, sufficient of the organic liquid is employed to dissolve all of the hydrocarbon-insoluble material and the solution then allowed to cool and crystallize. Such slight amount of material insoluble in the alcohol mixture as may be encountered can be removed readily by filtering the hot solution.

The solution is allowed to cool to ambient temperature and filtered. The dark violet crystals of *cis*-bixin thus obtained can be washed with a small amount of fresh solvent and then dried. The dry crystals usually contain from about 80 per cent to about 90 per cent of *cis*-bixin and are generally pure enough for most uses without further purification. They can be re-crystallized readily from an additional quantity of the same solvent mixture, if desired, *and cis*-bixin obtained readily having a melting point of about 185 degrees centigrade. The yield is generally from about 20 per cent to about 30 per cent of the crude extract obtained when fresh seeds are extracted.

The mother liquor from the *cis*-bixin crystals is distilled to volatilize substantially all of the solvent used in the crystallization step, care being taken that the residue remaining in the still is not heated above about 100 degrees centigrade. The residue thus obtained is cooled and is then in the form of a dark red, sticky resinous solid. It contains substantially all of the *trans*-bixin extracted from the seeds together with various other oxygen-containing carotenoids. The product is insoluble in aliphatic hydrocarbons and other non-polar organic liquids, but is soluble in cold polar liquids, such as the alcohols, especially after preliminary warming. It is useful as a coloring material and contains a considerable proportion of the biological activity of the crude extract. It is to be noted that the resinous solid contains substantially all of the crude extract not isolated as the oleoresinous liquid or as crystallized *cis*-bixin and that there is no loss of the crude extract encountered in preparing the three fractions thereof by the procedures just described.

The process can be varied somewhat, especially in its initial steps. One such variation comprises extracting the fresh dry seeds first with a non-polar organic liquid, preferably with an aliphatic hydrocarbon, rather than with a polar organic liquid or a mixture thereof with a non-polar liquid. The extraction of the dry seeds with a non-polar organic liquid is carried out substantially as described above for a polar liquid. In this instance, however, the insoluble material is not discarded but is saved for subsequent treatment. The liquid extract remaining after separating the insoluble material is

then heated at below about 100 degrees centigrade to volatilize the non-polar liquid and there is thus obtained an oleoresinous liquid residue substantially identical in properties and mount with that described previously.

The seed material containing substances insoluble in the non-polar organic liquid is then extracted with a polar organic liquid, e.g., with a mixture of equal parts by volume of ethanol and trichloroethylene, in substantially the same fashion as described for obtaining the crude extract referred to previously when the untreated seeds were extracted using this same liquid mixture. The residual seed material which is insoluble in the polar organic liquid is discarded after steaming to recover solvent. The hot filtrate is allowed to cool to cause crystallization of *cis*-bixin. The *cis*-bixin is recovered and the filtrate worked up to recover the resinous solid in a manner substantially identical with that described previously, for recovering these two substances from the portion of the crude extract which is insoluble in an aliphatic hydrocarbon. The proportions of *cis*-bixin and of the properties of these two substances are substantially identical with those obtained employing the procedure first described.

Certain other modifications of the process can be employed without departing from the spirit of the invention. Thus, the solution of crude extract obtained by extracting the whole seeds with a polar organic liquid can be allowed to cool and crystallize and a part of the *cis*-bixin recovered in more or less crude form at this point. The filtrate from the *cis*-bixin recovered in this way can then be evaporated and the residue worked up in the manner described to recover the oleoresinous liquid, the resinous solid and the balance of the *cis*-bixin. Other contemplated variations will be apparent in view of the disclosure set forth. It is desirable to carry out the entire process with as nearly complete exclusion of light and air as is convenient to avoid a partial decomposition of certain of the more sensitive components of the pulp which may otherwise occur.

Certain advantages of the invention are apparent from the following examples, which are given by way of illustration only and are not to be construed as limiting.

EXAMPLE 1

A mixture of 500 grams of unground, dry, ripe seeds of *Bixa orellana* from which the pulp had not been separated, 500 milliliters of 95 per cent ethanol and 500 milliliters of trichloroethylene was stirred and heated to boiling under reflux for about one-half hour. The mixture was allowed to cool to about 30°C. and the liquid decanted. This procedure was repeated three times and the insoluble material consisting of the seeds and disintegrated pulp residue, which was substantially free of carotenoids and pigments, was discarded.

The decanted liquids were combined and evaporated in a still until substantially all of the ethanol and trichloroethylene had distilled. The temperature of the residue in the still was kept below about 80°C. The still residue consisted of about 32 grams of dark red, semi-crystalline, pasty crude extract.

The still residue was then mixed thoroughly with about 175 milliliters of commercial petroleum ether having a boiling range of approximately 70 to 80°C. and the mixture allowed to stand over night. The petroleum ether solution was decanted and the residue washed again with about 100 milliliters of petroleum ether and saved for further treatment. The two decanted portions of petroleum ether solution were filtered to remove a slight amount of suspended insoluble material and the petroleum ether then distilled. The distillation was finished *in vacuo* so as to remove substantially all of the petroleum ether without heating the still body to a temperature higher than about 80°C. There was thus obtained a fraction of the crude extract consisting of 16.4 grams of a viscous oleoresinous, orange-red liquid having a pleasant, fruity odor. The oleoresinous liquid was substantially free of both *cis*- and *trans*-bixin and was completely soluble in petroleum ether and in a mixture of equal parts by volume of 95 per cent ethanol and trichloroethylene.

The residue which was insoluble in petroleum ether was dissolved in a boiling mixture of equal volumes of 95 per cent ethanol and trichloroethylene and the hot mixture filtered to clarify it and the filtrate allowed to cool to room temperature. The crystalline *cis*-bixin which separated slowly was recovered by filtering and washing with a small amount of the mixture of ethanol and trichloroethylene. The crystals after drying consisted of 9.4 grams of *cis*-bixin in the form of deep violet crystals. The purity of the material was about 89 per cent. Re-crystallization of this product from a mixture of equal volume of ethanol and trichloroethylene gave a product melting at 195.9°C. (corr.) and consisting of 98 per cent pure *cis*-bixin.

The filtrate from the crystals of *cis*-bixin was distilled to remove substantially all of the ethanol and trichloroethylene, being careful to avoid a still temperature higher than about 80°C. The residue consisted of 3.7 grams of a dark resinous solid which contained most of the *trans*-bixin present in the seeds originally extracted. The product was soluble in a cold mixture of equal volumes of ethanol and trichloroethylene but was insoluble in cold petroleum ether. The product was practically odorless.

EXAMPLE 2

500 grams of dry, ripe seed of *Bixa orellana* was extracted three times with 1 liter portions of hot petroleum ether having a boiling range of about 70° to 80°C. The combined filtered extracts were distilled to remove substantially all of the petroleum ether, the distillation being finished under vacuum to avoid heating the still residue higher than about 80°C. There was thus obtained an oleoresinous liquid similar in amount and in all other respects to the oleoresinous liquid obtained in Example 1.

The entire portion of the seed material which was insoluble in petroleum ether was extracted with three separate 1,000 milliliter portions of a boiling mixture of equal parts by volume of 95 per cent ethanol and trichloroethylene. The insoluble portion remaining undissolved in the mixture was substantially free of carotenoids and pigment materials and was discarded. The three extracts were combined and filtered hot to remove all traces of insoluble substances and the hot solution allowed to cool and crystallize. This mixture was worked up according to the procedure described for the hydrocarbon-insoluble residue of Example 1 and there were thus recovered crystallized *cis*-bixin and a resinous solid, each similar in amount and in all other respects to the crystalline *cis*-bixin and resinous solid obtained in Example 1.

EXAMPLE 3

The procedure of Example 1 was repeated except that the extraction of the dry seeds of *Bixa orellana* was carried out using a mixture of equal parts by volume of acetone and trichloroethylene and the extraction was carried out at a temperature of about 40°C. The crude extract was recovered by evaporating the acetone and trichloroethylene under vacuum so that the temperature of the still residue did not exceed about 40°C. The crude extract thus obtained was then worked up in the same manner as the crude extract of Example 1 and entirely similar results were obtained.

In still other experiments following the procedure of Example 1 for separation of the crude extract into fractions, the extraction of the seed was carried out using acetone or chloroform alone to obtain the crude extract. The products obtained were analogous in all respects to those obtained in Example 1.

What I claim is:—

1. A process for separating extractable substances occurring naturally in the seed pulp of *Bixa orellana* which comprises treating dry whole seeds of *Bixa orellana* or the crude extract obtained after evaporating the solvent from the filtrate obtained after heating the dry whole seeds with a polar organic liquid or a mixture of a polar organic liquid and a non-polar organic liquid and removing the insoluble portion, with an unheated non-polar organic liquid, separating the insoluble constituents, heating the solution to vaporise the non-polar liquid to leave a substantially bixin-free viscous oleoresinous liquid soluble in both non-polar and polar organic liquids, dissolving the non-polar organic liquid insoluble constituents in a heated polar organic solvent or a heated mixture of a polar solvent and a non-polar organic solvent therefor, cooling the solution to cause crystallisation of the *cis*-bixin, separating the crystallised *cis*-bixin, evaporating the solvent from the crystal-free solution to leave a resinous solid product soluble in cold polar organic liquids and substantially insoluble in non-polar organic liquids and comprising a major portion of the *trans*-bixin originally present in the crude extract.

2. A method as claimed in Claim 1 wherein the mixture of polar and non-polar organic solvent used in treating the dry seeds is a mixture of an aliphatic alcohol and a haloaliphatic hydrocarbon.

3. A process as claimed in Claim 1 or 2 wherein the dissolving of the non-polar organic liquid insoluble constituents in a polar solvent and the evaporation of the polar organic liquid from the *cis*-bixin crystal-free solution is carried out at a temperature below about 100°C.

4. A method as claimed in any preceding claim wherein the non-polar organic liquid insoluble constituents are heated in an alcohol boiling below about 100°C.

5. A method as claimed in Claim 1 wherein the non-polar organic liquid used in treating the seeds or the crude extract is an aliphatic hydrocarbon and the non-polar organic liquid insoluble constituents are heated with a mixture of an alcohol and a haloaliphatic hydrocarbon.

6. A method as claimed in Claim 5 wherein the liquid aliphatic hydrocarbon has a boiling point below about 100°C.

7. A process as claimed in any preceding claim wherein the entire process is carried out below about 100°C.

8. A method as claimed in Claim 5 wherein the mixture of the alcohol and the haloaliphatic hydrocarbon employed comprises substantially equal parts by volume of ethanol and trichloroethylene.

9. A process for recovering *cis*-bixin substantially free from *trans*-bixin which comprises treating the crude extract comprising the carotenoids and wax pigments occurring naturally in the seed pulp of *Bixa orellana* obtained either by separating the non-polar organic liquid insoluble constituents from dry whole seeds of *Bixa orellana* by treatment with a non-polar organic solvent, or by separating the non-polar organic liquid insoluble constituents from the extract ob-

tained after evaporating the solvent from the filtrate obtained by treating the dry whole seeds with a polar organic liquid or a mixture of a polar and non-polar organic liquid and after removing the insoluble portion and treating the latter with a non-polar solvent, with a hot mixture of an alkanol and a halo-aliphatic hydrocarbon, cooling the solution and recovering crystallised *cis*-bixin substantially free from *trans*-bixin.

10. A process as claimed in Claim 9 wherein the mixture of the alkanol and halo-aliphatic hydrocarbon employed is a mixture of approximately equal parts by volume of ethanol and trichloroethylene.

11. A process for separating extractable substances occurring naturally in the seed pulp of *Bixa orellana* into a plurality of separate products distinct from one another substantially as herein described with reference to the examples.

12. Extractable carotenoid and pigment substances occurring naturally in *Bixa orellana* when separated by a process as claimed in any of Claims 1 to 11.

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